REMARKS/ARGUMENTS

Claims 1-8 and 10-21 are currently pending in the application. In the Office Action mailed February 2, 2005, previous rejections under 35 U.S.C. §112, second paragraph, and under 35 U.S.C. §103, have been withdrawn. New rejections have been issued. Claims 1-8 and 10-21 now stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite, and under 35 U.S.C. §102(b), as being anticipated by Allex et al. (1977).

In response to the outstanding rejections, claims 1, 5 and 18, 19, 20 and 21 have been amended so as to more particularly define the scope of the present invention. For the reasons set forth below, Applicants respectfully request reconsideration of the rejection, and withdrawal of the rejections.

Summary of Claimed Invention

The present invention is directed to a method for alignment of a plurality of data traces indicative of the positions of a plurality of nucleic acid base types in a target nucleotide sequence. Data traces are graphical representations of fluorescent signals of nucleic acid chain termination fragments. Typically, a single data trace is generated for each of the four different classes of chain termination fragments (i.e., terminating with the base types A, G, C, and T). *Alignment of data traces* is the comparison of one data trace with another data trace, and is performed for the purpose of establishing the correct order of nucleic acid base signals of one data trace relative to another data trace. Correct alignment of data traces is therefore necessary for (and performed prior to) establishing the correct order of bases in a nucleic acid sequence (referred to as "base calling") so as to generate a nucleotide sequence. *Alignment of nucleotide sequences*, on the other hand, is the comparison of one nucleic acid sequence with another nucleic acid sequence, and is performed for the purpose of comparing or confirming one nucleotide sequence with another. Thus, alignment of nucleotide sequences inherently requires that two nucleotide sequences already have been generated by alignment of data traces and base calling. Alignment of data traces thus precedes alignment of nucleotide sequences, and it is therefore evident that the two are distinct and separate steps.

The present invention is directed to a method of aligning *data traces*. According to the claimed method, one or more alignment points in a data trace are selected. The alignment point may be a single or multiple internal alignment point based on a highly conserved region. For

example, the alignment points may be selected from among the (i) primer peak, (ii) the full-length peak, and (iii) internal peaks representing highly conserved bases. The internal peak alignment points may also be heterogeneous nucleotide multiplets. Reference position numbers are assigned to these alignment points, reflecting the known position of the alignment point relative to the rest of the sequence. These reference position numbers are used as a reference point in the next step when comparing position numbers that are assigned to peaks in the data traces. A sequence position number is assigned to each peak in the experimental data trace so as to maximize the number of times that the sequence position number of the data trace is assigned to the same base as the reference position number of the alignment points. Optionally, the claimed method may also include a step of determining the average peak spacing interval between alignment points and assigning a sequence position number to peaks occurring at the intervals. The data traces are then aligned based on the assigned sequence position numbers.

The method of the present invention described above has been shown to result in significantly improved accuracy of base-calling, particularly where there is gross misalignment problems. Prior art methods of base calling are inherently incapable of processing data traces with gross misalignment between them. It is not uncommon, particularly when base calling is required in conjunction with the use of 2-color sequencers that use up to 4 traces obtained from different physical lanes of the slab gel or different capillaries for there to be a shift between traces exceeding 20–30 bases. Alignment of data traces with such shift without *a priori* information is highly complicated, and algorithms used prior to the present invention are not always capable of successfully resolving such data traces. The method of the present invention, which utilizes stable conserved peaks (such as primer peaks, final full-length peaks and/or conserved regions of sequence), however, has been shown to enable successful and accurate alignment of data traces having initial misalignment as high as 150 bases (see, e.g., Figures 10 and 11 of the specification), which is a highly significant improvement over the prior art that was not expected, nor could have been predicted *a priori*. This is a surprising and unanticipated result, that highlights the novelty and non-obviousness of the present invention.

Rejections under 35 U.S.C. §112, second paragraph

The Examiner has rejected claims 1-8 and 10-21 under 35 U.S.C. §112, second paragraph, as being indefinite. Specifically, in paragraph 8, the Examiner states that the

limitation of "set of three or more alignment points" is vague and indefinite, in that "it is not clear as to whether step (c) is a realigning of the already aligned points selected in step (a)." Applicant respectfully notes that the term "alignment points" does not grammatically imply that the points have been previously aligned (the proper grammatical construction for such an interpretation would be "aligned points"). Rather, the term "alignment points," as described in the specification merely refers to points that are "used as reference points in the alignment of the traces" (page 6, lines 14-15). The specification therefore makes clear that the alignment points are points that are subsequently used in the alignment step (c), and not points that were previously aligned. In view of the fact that the grammatical construction of the term "alignment points" is correct as it stands, and further in view of the fact that the specification in any event expressly confirms that interpretation, Applicant submits that there is no ambiguity in the claim language. Applicant therefore respectfully requests that the rejection be withdrawn.

In paragraph 9, the Examiner further notes that in claims 1, 5 and 10 the term "the alignment point" lacks antecedent basis "because all previous citations are directed to plural points." Applicant acknowledges and concurs with the Examiner's point, and has amended claims 1 and 5 so that the term "point" is used consistently in singular form. Specifically, Applicant has amended claim 1 to recite "assigning to each ~~said~~ alignment ~~points~~ point a reference position number reflecting the relative position of the alignment point with respect to the sequence as a whole." The language of Claim 10 already uses the term "point" in singular form (i.e., "assigning to *each* alignment *point* a reference position number reflecting the relative position of the alignment *point*..."), so no amendment is necessary. Applicant submits that the above amendments are responsive to the issue noted by the Examiner, and respectfully requests reconsideration and withdrawal of the rejection.

In paragraph 10, the Examiner notes that the phrase "nucleotide base type" is vague and indefinite "because it is unclear whether the limitation of 'nucleotide base type' is directed to natural or synthetic nucleotide base type" and further because "said limitation could reasonably be construed as being directed to the RNA or DNA nucleotide base type." Applicant respectfully submits that the phrase "nucleotide base type" is not vague or indefinite. As clearly set forth in the specification, the phrase "nucleotide base type" is a well-known term of art, referring to the nucleic acid bases adenine (A), cytosine (C), guanine (G), or thymine (T), of which those skilled in the art understand include natural and synthetic analogs. Applicant respectfully notes that the

utility and operability of the present invention does not depend on whether the nucleotide base type is natural or synthetic. Indeed, it is completely irrelevant to the present invention. The present invention is instead directed to a method of aligning nucleotide sequence traces (irrespective of whether the data trace is derived from a natural or synthetic polynucleotide sequence). Consequently, there is no rational or scientific basis for asserting that the nature of the nucleotide base is relevant to the utility or operability of the invention or requires further limitation to one or the other. In short, the present invention is directed to a method of aligning sequence traces, regardless of the particular source of the nucleotide sequence. In view of the fact that the phrase "nucleotide base type" is a well-known term of art, and the particular *source* of the nucleotide base does not constitute a point of novelty of the invention, Applicant submits that the claims provide adequate written description of the metes and bounds of the invention. Applicant accordingly requests that the above rejection be withdrawn.

Finally, in paragraph 11, the Examiner states that the singular phrase "spacing interval" does not constitute adequate antecedent basis for the plural "said intervals." Applicant acknowledges the Examiner's point, and has amended claims 18, 19, 20 and 21 to clarify the noted ambiguity. Specifically, Applicant has amended claim 18 to recite the step of "determining the average peak spacing interval between each of the alignment points and assigning sequence position numbers to peaks occurring at each of said intervals." Similar amendments have been made to claims 19, 20 and 21. It should be noted that the plural form of "points" is necessary in this case, since the claim is defining the spacing interval between *two points*. As recited in claim 1, there exist "three or more alignment points." Because there are a plurality of alignment points, there is necessarily a "spacing interval" between each of the three alignment points. Applicant submits that the above amendment clarifies that two alignment points define a spacing interval between the two alignment points, and that three alignment points define at least two spacing intervals (three, if the spacing interval between the first and third is included). In view of the above amendment, Applicant submits that the claim terminology is consistent and there is no ambiguity with respect the antecedent basis of the term "intervals." Applicant accordingly request that the rejection be reconsidered and withdrawn.

Rejection under 35 U.S.C. §102(b)

The Examiner has also rejected claim 1-8 and 10-13 under 35 U.S.C. §102(b), as being anticipated by Allex et al. (1997). The Allex et al. reference discloses methods for increasing accuracy of *consensus calling* in DNA fragment assemblies of *multiple sequences* by comparing *previously made base calls* of the various fragments being aligned, and also by comparing the *quality* of the fluorescent trace data for each base call at a specified base location (i.e., the visual characteristics of the base call, such as the shape and intensity, which are assigned a weighted score). The "consensus" base, from among the various fragments being aligned, is the base having the greatest sum of evidence or weight.

For the following reasons, Applicant submits that the Allex et al. reference does not teach or suggest the present invention. As noted above, the present invention is directed to a method for aligning data traces for the purpose of *base calling*, by aligning a plurality of data traces indicative of the positions of a plurality of nucleic acid base types (A, G, C or T) in a target nucleic acid sequence. As noted above, the process of nucleic acid sequencing involves generating a plurality of data traces (for example, one for each of the base types A, G, C and/or T). In order to determine the nucleic acid sequence of given polynucleotide, each of the data traces for A, G, C and T must be properly aligned, so as to reflect the correct order of the different nucleic acid base types relative to each other. The combined, or "aligned," sequence is then read to determine the correct order of bases. This is referred to as "base calling." The present invention relies upon the conserved features of a nucleotide sequence to provide calibration markers for alignment of the data traces. Specifically, three or more alignment points are selected in each data trace, which are associated with a reference position number indicative of the position of the alignment point in the sequence as a whole. The data traces are aligned so as to match the reference position number of the alignment points and the sequence position number with the same base, thereby enabling accurate base-calling.

The present invention is distinguishable over the Allex et al. reference because the present invention is directed to a method of aligning data traces for the purpose of *base calling*, while Allex et al. relates to a method for aligning nucleotide sequences for the purpose of *consensus calling*. The distinction is significant. Base calling relates to the process of making the initial base call of a particular sequence. In contrast, consensus calling relates to the process

of comparing two different nucleotide sequences (which were previously determined using a process of base calling) to obtain a consensus as between the two sequences. Thus, the present invention aligns *individual* data traces for each base type (A, G, C or T) in order to determine the nucleotide base type at each position of the sequence, and thereby determine the sequence of the single polynucleotide. The method of Allex et al. begins with *multiple* existing polynucleotide sequences for which the nucleotide sequence has already been determined (i.e., base calling has already been performed), and compares and aligns the bases of multiple polynucleotide sequences at each nucleotide base position, so as to determine which base at a particular location is the consensus among the multiple sequences. Because the method of Allex et al. compares two complete nucleotide sequences, the method inherently compares *multiple* nucleotide bases assigned to the *same* base position. In contrast, because the method of the present invention compares two or more data traces (each of which represent only a single base type), only *one* nucleotide base can theoretically be assigned to any given position, and no "comparison" is made between different sequences. For the above reasons, Applicant submits that Allex et al. does not teach or suggest the claimed method of aligning data traces for the purpose of base calling.

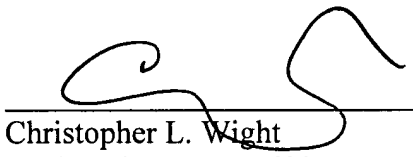
Notably, the Allex et al. reference also does not disclose other limitations of the claims. For example, Allex et al. does not disclose the use of "three or more alignment points," selected from "a primer peak," or "a full-length peak." Allex et al. also does not disclose the use of "one or more internal peaks associated with internal bases that are highly conserved in the target nucleic acid." Furthermore, Allex et al. does not disclose "assigning a sequence position number" that "maximizes the number of times that the sequence position number and the reference position number are assigned to a base of the same type." In view of the fact that Allex et al. is non-analogous art, but also fails to teach or suggest the above claim limitations, Applicant respectfully submits that Allex et al. does not teach or make obvious the present invention and requests that the above rejection be withdrawn.

CONCLUSION

In response to the Examiner's rejections, Applicant has amended the claims to more particularly point out and define the claimed invention. Applicant has also provided a detailed explanation of the differences between the present invention and the cited prior art. In view of the differences between the cited art relied on by the Examiner and the limitations recited in the claims, the cited art neither teaches nor suggests the claimed invention.

For the above reasons, Applicant respectfully submits that the grounds of rejection have been obviated and requests allowance of the claims.

Respectfully submitted,



Christopher L. Wight
Registration No. 31,680
Holland & Hart LLP
555 17th Street, Suite 3200
Denver, CO 80201-8749
Telephone: (801) 595-7823
Fax: (801) 364-9124

CLW/jml
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